

# 8EHQ-0303-15196

# **Acetophenone Task Force**

1203 Nineteenth Street, N.W., Suite 300 Washington, D.C. 20036-2401

March 5, 2003

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Via Certified Mail Return Receipt Number 7002 1000 0005 0845 2695

TSCA Section 8(e) Coordinator Document Control Officer (MC-7407) Office of Pollution Prevention and Toxics U.S. Environmental Protection Agency Ariel Rios Building 1200 Pennsylvania Avenue, N.W. Washington, DC 20460-0001



Toxic Substances Control Act -- Section 8(e) Re:

Dear TSCA Section 8(e) Coordinator:

This letter supplements the September 9, 2002, and September 26, 2002, letters from the Acetophenone Task Force (ATF), c/o Mr. William J. Moffatt, JLM Chemicals, Inc., 3350 West 131st Street, Blue Island, Illinois 60406-2365, to the U.S. Environmental Protection Agency (EPA) regarding its submission, pursuant to Section 8(e) of the Toxic Substances Control Act (TSCA) of preliminary results of an Organization for Economic Cooperation and Development (OECD) \$\frac{1}{42}\$2 Combined Repeated Dose Toxicity Study and Reproduction/Developmental Screening Study in Sprague-Dawley Rats with Acetophenone (CAS No. 98-86-2). The study was conducted as part of the ATF's sponsorship of acetophenone in the OECD Screening Information Data Set (SIDS). Program and has now been issued in final. Although there is no new information regarding this chemical from this study that is considered to present a substantial risk to human health or the environment under TSCA Section 8(e) that was not already provided in our September 9, 2002, and September 26, 2002, letters, the ATF is submitting to EPA the final version of the study.

Contain No Lu.

The ATF is comprised of the following companies: JLM Chemicals, Inc. and Aceto Corporation.

# **Acetophenone Task Force**

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TSCA Section 8(e) Coordinator March 5, 2003 Page 2

If you have any questions, please call Lynn L. Bergeson, legal counsel to the ATF, at (202) 557-3801, or e-mail her at lbergeson@lawbc.com.

Sincerely, ACETOPHENONE TASK FORCE

By: William J. Moffatt

William J. Moffatt

Chair

Attachment

cc: Acetophenone Task Force (w/o attachment) (via e-mail)

### A COMBINED REPEATED DOSE TOXICITY STUDY AND REPRODUCTION/DEVELOPMENTAL SCREENING STUDY IN SPRAGUE DAWLÉY RATS WITH ACETOPHENONE

FINAL REPORT Volume 1 of 2



# A COMBINED REPEATED DOSE TOXICITY STUDY AND REPRODUCTION/DEVELOPMENTAL SCREENING STUDY IN SPRAGUE DAWLEY RATS WITH ACETOPHENONE

FINAL REPORT Volume 1 of 2

**Study Director** 

Bjorn A. Thorsrud, Ph.D.

Study Completed on

January 13, 2003

**Performing Laboratory** 

Springborn Laboratories, Inc. (SLI)
Ohio Research Center
640 North Elizabeth Street
Spencerville, OH 45887

SLI Study No.

3546.1

Submitted to:

Acetophenone Task Force 1300 Eye Street, N.W. Suite 1000 West Washington, D.C. 20005

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# 1. COMPLIANCE STATEMENT

This study was conducted in compliance with the Principles of Good Laboratory Practice as described by the OECD [Revised ENV/MC/CHEM(98)17] and SLI's Standard Operating Procedures.

Bjorn A. Thorsrud, Ph.D.

Study Director

Springborn Laboratories, Inc.

Date 1.13.03

# 2. QUALITY ASSURANCE STATEMENT

This study was inspected by the Quality Assurance Unit and reports were submitted to management and the Study Director in accordance with SLI's Standard Operating Procedures as follows:

<u>Phase</u>	<u>Date</u>
Protocol Review	03/06/01
Protocol Amendment Review	04/12/01, 09/28/01
Retention Sample	06/05/01
Animal Receipt	08/28/01
Homogeneity and Stability Analyses	09/21/01
Randomization	10/02/01
Body Weights	10/02/01
Clinical Observations	10/02/01, 10/03/01
Dose Preparation	10/02/01
Dose Concentration Analyses	10/03/01
Dosing	10/03/01
Food Consumption	10/10/01
Functional Observation Battery	10/10/01
Cohabitation	10/17/01
Detection of Copulatory Plug/Sperm	10/18/01
Blood Collection	11/05/01
Hematology/Coagulation Evaluations	11/05/01
Necropsy	11/05/01, 11/13/01
Organ Weights	11/05/01
Biochemistry Evaluations	11/06/01
Pup Viability/External Examination/Sex	
Determination	11/09/01
Parturition	11/09/01
Pup Body Weights	11/12/01
Staining and Tissue/Slide/Block	
Accountability and Histology	01/18/02, 01/20/02,
	04/15/02
Data Audits	06/07/01, 06/11/01,
	12/26/01, 01/04/02,
D (D )	01/07/02
Draft Report Review	05/03/02, 11/05/02
Final Report Review	01/13/03

Reports Submitted to Study Director and Management

03/07/01, 06/05/01, 06/07/01, 08/28/01, 09/21/01, 10/01/01, 10/03/01, 10/10/01, 10/12/01, 10/18/01, 11/09/01, 11/13/01, 11/28/01, 12/27/01, 01/07/02, 01/08/02, 01/21/02, 02/15/02, 05/03/02, 11/05/02 01/13/03

The final report has been reviewed to assure that it accurately describes the materials and methods, and the reported results accurately reflect the raw data.

Jody R/Heitmeyer, B.S. / Quality Assurance Auditor

Anita M. Bosau, RQAP-GLP

Senior Director, Compliance Assurance

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#### 6. SUMMARY

This study was conducted to: (1) provide initial information on the repeated-dose systemic toxicity of Acetophenone, with emphasis on potential neurological effects, and (2) serve as a screening study for potential reproductive and developmental effects in male and female rats. This study was not designed to provide a complete assessment of all aspects of reproduction and development; but to offer a limited means of detecting postnatal manifestations of prenatal exposure, as well as effects induced during limited postnatal exposure. The study consisted of a toxicity study and a reproduction/developmental screening study. Experimental designs for the toxicity study and the reproduction/ developmental screening study are provided below:

	-,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,		Toxicity Stu	idy		
		f Adult mals	Dosage	Dosage Level	Dosage Conc.	Dosage Volume
Group	Male	Female	Material	(mg/kg/day)	(mg/mL)	(mL/kg)
1	10	5	Corn Oil	0	0	2
2	10	5	Acetophenone	75	37.5	2
3	10	5	Acetophenone	225	112.5	2
4	10	5	Acetophenone	750	375	2

Note: After a minimum of 14 days of treatment, males were cohabited with females in the reproduction/developmental screening study.

The test article was administered once daily by oral gavage for a minimum of four weeks, up to and including the day prior to scheduled euthanasia. Cage-side observations for overt signs of toxicity were performed once daily within approximately one-half hour to two hours following dosing. Detailed clinical observations were performed a minimum of weekly and on the day of scheduled euthanasia. An abbreviated functional observation battery was performed for all males and females beginning on day -1 and weekly thereafter. Following 28 days of treatment, an abbreviated functional observation battery was performed for 10 males in groups 1-4. Manipulative tests were performed for 10 males in groups 1 and 2 and, 5 males in groups 3 and 4. Motor activity was conducted in 5 males in groups 1-4. A full functional observation battery was performed on 5 females from each group. Individual body weights were recorded on days 0, 3, 7, 12, 16, 20, 23, 27 and 30. A final body weight was recorded prior to scheduled euthanasia (day 33/34). Individual food consumption was recorded on the same days as body weights (except for males during cohabitation with females in the reproduction/developmental screening study). Blood samples were collected from five males and five females from each group on the day of scheduled euthanasia (day 33/34) for evaluation of selected hematology, coagulation and clinical chemistry parameters. All animals were subjected to a complete gross necropsy examination at scheduled euthanasia (day 33/34). Fresh organ weights were recorded and selected tissues and organs were preserved from all animals. All tissues and organs collected at necropsy from five males and five females from each group and gross lesions from all animals were examined microscopically.

	Reproduction/Developmental Screening Study						
Group	No. of Females	Dosage Material	Dosage Level (mg/kg/day)	Dosage Conc. (mg/mL)	Dosage Volume (mL/kg)		
1	10	Corn Oil	0	0	2		
2	10	Acetophenone	75	37.5	2		
3	10	Acetophenone	225	112.5	2		
4	- 10	Acetophenone	750	375	2		

The test article was administered once daily (seven days a week) by oral gavage for a minimum of 14 days prior to mating and continuing through lactation day 3. Cage-side observations for overt signs of toxicity were performed once daily within approximately one-half hour to two hours following dosing. Detailed clinical observations were performed a minimum of weekly until evidence of mating was detected, daily during gestation and lactation and on the day of scheduled euthanasia. Individual body weights were recorded on days 0, 3, 7 and 12 prior to mating, on gestation days 0, 7, 14 and 20 and on lactation days 1 and 4. Individual food consumption was recorded on the same days as body weights (except during cohabitation). After a minimum of 14 days of treatment, females were cohabited with males from the same treatment group in the toxicity study. Following parturition, pup viability, sex determinations, observations and body weights were recorded at specified intervals during lactation days 0 and 4. Surviving females and their pups were euthanized and necropsied on lactation day 4. Selected tissues and organs were collected from the females at necropsy and retained in 10% neutral buffered formalin for possible future histopathological evaluation.

No mortality occurred in the toxicity study. Clinical signs were observed for males and females in the 225 and 750 mg/kg/day groups. The clinical signs included urine stain, predose salivation and postdose salivation in the 225 and 750 mg/kg/day groups, and postdose wobbly gait and postdose urine stain in the 750 mg/kg/day group. Remarkable clinical signs in the 75 mg/kg/day group were limited to a single incidence of postdose salivation in one female. Mean forelimb grip strength and mean motor activity of males in the 750 mg/kg/day group were statistically lower than controls on day 29. No other toxicologically meaningful differences were noted in the functional observation battery.

Statistically significant body weight loss occurred for males in the 750 mg/kg/day group during days 0-3. An apparent body weight loss was noted in all dose groups for both male and female animals during days 12-16 and this was followed by a compensatory rebound in weight gain the following body weight change interval (days 16-20). The definitive reason for this occurrence could not be determined, but it may be related to the initiation of mating. There were no statistically significant differences in mean body weights; however, mean body weights of males in the 750 mg/kg/day group were approximately 5-10% lower than controls on days 3-30. No other toxicologically meaningful differences in mean body weights or body weight change were noted during the study.

Mean food consumption of males and females in the 750 mg/kg/day group was statistically lower than controls during study days 0-3. In addition, mean food consumption appeared to be slightly, but not statistically lower than controls for females in the 225 mg/kg/day group

during days 0-3 and males in the 750 mg/kg/day group during days 16-30. No other toxicologically meaningful differences in mean food consumption were noted during the study.

There were no toxicologically meaningful differences between the control, 75, 225 and 750 mg/kg/day groups in the hematology and coagulation parameters evaluated at study termination. With the possible exception of higher mean total protein, calcium and cholesterol for males in the 750 mg/kg/day group and higher mean cholesterol for females in the 750 mg/kg/day group, there were no toxicologically meaningful differences in the biochemistry parameters evaluated. The toxicological significance of the higher total protein, calcium and cholesterol values is not clear; however, the mean values for these parameters were outside the range of SLI historical control data. There was a common finding to both male and female animals that included a dose-related increase in total protein, albumin and globulin that appeared to correlate with a dose-related increase in liver weight. These changes may be related to a compensatory reaction to the test article, possibly due to its metabolism.

No remarkable gross necropsy findings were noted in the control, 75, 225 or 750 mg/kg/day groups and there were no toxicologically meaningful differences in absolute or relative organ weights between the groups. No toxicologically significant test article-related microscopic lesions were noted in any of the tissues examined. A nondose-related, minimal to mild hyaline droplet nephropathy was observed for males in the 75, 225 and 750 mg/kg/day groups; however, this finding is not considered toxicologically significant for humans.

No mortality occurred for the F0 females in the reproduction/developmental screening study. Clinical signs were observed for F0 females in the 225 and 750 mg/kg/day groups. The clinical signs included urine stain, predose salivation, postdose feces small in size and postdose salivation in the 225 mg/kg/day group, and decreased activity, skin pale in color, unkempt appearance, rough coat, postdose dark material around nose, postdose few feces, predose and postdose salivation and postdose wobbly gait in the 750 mg/kg/day group. In addition, one female in the 750 mg/kg/day group appeared to have prolonged parturition. Remarkable clinical signs for females in the 75 mg/kg/day group were limited to a single incidence of urine stain in two females.

Mean body weight change of F0 females in the 750 mg/kg/day group was statistically lower than controls during gestation days 0-7. There were no statistically significant differences in mean body weights; however, mean body weight of F0 females in the 750 mg/kg/day group was slightly (approximately 6%) lower than controls on gestation day 7. No other toxicologically meaningful differences in mean body weights or body weight change were noted during the study.

Mean food consumption of F0 females in the 225 and 750 mg/kg/day groups was statistically lower than controls during days 0-3. In addition, mean food consumption appeared to be slightly, but not statistically lower than controls for F0 females in the 750 mg/kg/day group during gestation days 0-7 and lactation days 1-4. No other toxicologically meaningful differences in mean food consumption were noted between the control, 75, 225 and 750 mg/kg/day groups.

The F0 female mating and fertility indices and mean gestation lengths were comparable between the control group, 75, 225 and 750 mg/kg/day groups. The mean number of F1 pups delivered was comparable in the control, 75, 225 and 750 mg/kg/day groups. However, the live birth index (number of liveborn pups/total number of pups delivered) was statistically lower in the 750 mg/kg/day group compared to controls, and the number of stillborn pups in the 750 mg/kg/day group was statistically higher than controls.

The number of F1 pups dying, missing and/or cannibalized during lactation days 1-4 and the number of litters with total litter loss in the 750 mg/kg/day group were statistically higher than controls and the number of surviving pups on lactation day 4 in the 750 mg/kg/day group was statistically lower than controls. The mean live pups/litter in the 750 mg/kg/day group was slightly, but not statistically lower than controls on lactation day 0 and statistically lower than controls on lactation days 1-4. Mean F1 pup weights in the 750 mg/kg/day group were statistically lower than controls on lactation days 1 and 4. F1 pup viability and mean F1 pup weights in the 75 and 225 mg/kg/day groups was comparable to controls during lactation days 0-4. The F1 pup sex ratio was comparable between the control, 75, 225 and 750 mg/kg/day groups on lactation days 0 and 4.

Remarkable F1 pup observations included desquamation in the 225 and 750 mg/kg/day groups and a low incidence of gasping and skin pale in color, cool to the touch, skin with shiny appearance and skin appears tight – restricts movement in the 750 mg/kg/day group. No remarkable F1 pup observations were noted in the 75 mg/kg/day group.

Remarkable gross necropsy observations for F1 pups stillborn included a single incidence each of cleft palate and edema, increased incidences of atelectasis and milk not present in the stomach, and dermal hypoplasia in the 750 mg/kg/day group. Remarkable gross necropsy findings for F1 pups born alive but found dead during lactation days 0-4 included scabbing, desquamation, dermal hypoplasia and an increased incidence of milk not present in the stomach in the 750 mg/kg/day group. In addition, autolysis was observed for 22 F1 pups found dead in the 750 mg/kg/day. No remarkable gross necropsy findings were noted for F1 pups stillborn or found dead in the 75 and 225 mg/kg/day groups. Remarkable gross necropsy findings for F1 pups at scheduled euthanasia (lactation day 4) included scabbing and desquamation in the 225 and 750 mg/kg/day groups. No remarkable gross necropsy findings were noted for surviving F1 pups in the 75 mg/kg/day group.

Remarkable gross necropsy findings in F0 females were limited to two females in the 750 mg/kg/day group with total litter loss that had pup tissue mixed with ingesta in the stomach. No other remarkable findings were noted at necropsy for F0 females in the control, 75, 225 or 750 mg/kg/day groups.

Based on the results of this study, a dosage level of 75 mg/kg/day was considered a no-observed-adverse-effect level (NOAEL) for systemic toxicity. Other than possible indications of central nervous system (CNS) depression, such as wobbly gait, reduced forelimb strength and decreased motor activity in the high-dose group, there was no evidence of more severe CNS effects in this study. Thus, a dosage level of 225 mg/kg/day was considered the NOAEL for neurological effects. As a result of a significantly decreased live birth index (increased rate of stillbirths), as well as decreased pup survival during the

lactation phase and a decreased mean pup body weight in the high-dose group, a dosage level of 225 mg/kg/day was considered a NOAEL for reproductive effects. However, the mating and fertility indices and mean gestation length for the test article-treated females appeared unaffected at dosage levels up to 750 mg/kg/day.

#### 7. INTRODUCTION

This report details the experimental procedures and results of a combined repeated dose toxicity study and reproduction/developmental screening study in Sprague Dawley rats with Acetophenone. The study was authorized by the Acetophenone Task Force, Washington, D.C., and was conducted at Springborn Laboratories, Inc. (SLI), 640 North Elizabeth Street, Spencerville, Ohio. This study is essentially a combination of OECD Guidelines 421 and 407, but uses fewer animals than if the two guidelines were conducted independently. The study was performed to partially fulfill EPA recommendations regarding specific testing necessary to meet a SIDS Dossier on Acetophenone. The study design was based on OECD Guideline 422, Combined Repeated Dose Toxicity Study with the Reproduction/ Developmental Toxicity Screening Test, adopted 22 March 1996. The Sprague Dawley rat was selected as the experimental model for this study since this species/strain has a proven sensitivity to a variety of agents and, therefore, provides a suitable animal model for testing chemicals and drugs for human risk assessment. In addition, the Sprague Dawley rat was recommended by the OECD for this study. Oral administration of the test article was selected since it is a potential route of human exposure and since it was recommended by the OECD for this study design. The protocol was signed by the Study Director on March 6, 2001 (GLP initiation date). The in-life phase of the main study was initiated on October 3, 2001, and concluded on November 25, 2001.

Prior to initiation of the toxicity and reproduction/developmental screening studies, a 14-day range-finding study was conducted in rats to aid in selection of dosage levels. The in-life phase of the range-finding study was initiated on April 20, 2001, and concluded on May 23, 2001. The experimental methods and results of the range-finding study are included in Appendix A.

#### 8. OBJECTIVE

This study was conducted to (1) provide initial information on the repeated-dose systemic toxicity of Acetophenone, with emphasis on potential neurological effects, and (2) serve as a screening study for potential reproductive and developmental effects in male and female rats. This study was not designed to provide a complete assessment of all aspects of reproduction and development; but to offer a limited means of detecting postnatal manifestations of prenatal exposure, as well as effects induced during limited postnatal exposure.

#### 9. MATERIALS AND METHODS

#### 9.1. Experimental Protocol

The study protocol, protocol amendments and protocol deviations are included in Appendix B.

#### 9.2. Test Article and Vehicle Control Material

#### 9.2.1. Test Article Receipt, Identification and Storage

The test article was received from JLM Chemicals, Inc., Blue Island, Illinois, and identified as follows:

Identification	Assigned	Physical	Receipt	Expiration
	SLI ID	Description	Date	Date
Acetophenone Lot No. R012-078	S01.001.3546	Slightly pale yellow liquid	03/20/01	None provided

A one gram retention sample of the test article was taken and stored at SLI. The retention sample and remaining test article were stored at ambient conditions. The purity of the test article was 98.80%. Documentation concerning chemical identification, purity, strength, stability and other required data was the responsibility of the Sponsor. A Certificate of Analysis, provided by the Supplier, is included in Appendix C.

#### 9.2.2. Vehicle Control Material Receipt, Identification and Storage

The vehicle control material used in the preparation of dosing mixtures and for administration to control animals was corn oil, NF. The corn oil was received at SLI, stored refrigerated and identified as follows:

Source	Lot Number	Assigned SLI ID	Physical Description	Receipt Date	Manufacturer's Expiration Date
Spectrum Chemical	QN0035	V01.019	Clear yellow liquid	03/15/01	02/28/02

#### 9.2.3. Dose Preparation

For each test article dose group, a specified amount of Acetophenone was weighed into a pre-calibrated beaker. A sufficient quantity of corn oil was added to the beaker to achieve the desired concentration and the mixture was stirred for 30 minutes. Each test article solution was prepared fresh weekly, dispensed into daily aliquots and stored in amber glass containers at ambient conditions. During each preparation, corn oil was dispensed into daily aliquots for administration to control animals. The physical state of the control and each dosing solution was recorded during each preparation. The vehicle was a clear yellow liquid

and each test article preparation was a clear yellow solution. All dosing solutions were prepared and dispensed under a fume hood. The dosing solutions were stirred continuously prior to and during dosing.

#### 9.2.4. Homogeneity, Stability and Concentration Analyses

Prior to initiation of the toxicity and reproduction/developmental screening studies, homogeneity and stability analyses were performed on concentrations of the test article in the vehicle which encompassed the low- and high-dose concentrations. Homogeneity analyses were performed on duplicate samples taken from the top, middle and bottom of the two mixtures. Stability of the test article in the vehicle was evaluated on duplicate samples on day 0 and at 3 days and 10 days of room temperature storage. Concentration verification analysis was performed on the vehicle and each test article dosing solution prepared for study weeks 0, 2, 4 and 6. The results of the homogeneity, stability and concentration verification analyses are included in the SLI Analytical Chemistry Report included in Appendix C.

# 9.3. Animals and Animal Husbandry

Animal housing and care were based on the standards established by the Association for Assessment and Accreditation of Laboratory Animal Care, International (AAALAC) and the guidelines set forth in the Guide for the Care and Use of Laboratory Animals, NIH Publication No. 96-03, 1996.

#### 9.3.1. Animal Receipt, Identification and Housing

Forty-five male Sprague Dawley Crl:CD®(SD)IGS BR rats were received on August 28, 2001, from Charles River Laboratories, Inc., Raleigh, North Carolina, and sixty-five female Sprague Dawley Crl:CD®(SD)IGS BR rats were received on August 28, 2001, from Charles River Laboratories, Inc., St. Constant, Quebec. At the time of receipt, each rat was identified with a metal ear tag displaying a unique number. Animals were housed individually during acclimation and while on study (except during cohabitation) in suspended stainless steel cages. A cage card displaying the study, animal and group numbers, and sex was affixed to each cage. The cage cards were color-coded according to group number following randomization.

#### 9.3.2. Acclimation

The animals were examined upon receipt and daily thereafter during acclimation for signs of physical or behavioral abnormalities. General health/mortality checks were performed twice daily, in the morning and afternoon. Individual body weights were recorded on the day following receipt and prior to randomization on day -1. The animals were acclimated to the laboratory conditions for a period of 36 days prior to initiation of dosing.

#### 9.3.3. Diet and Drinking Water

PMI Certified Rodent Meal® #5002 (Purina Mills, Inc.) and municipal tap water were provided to each animal ad libitum. The feed was analyzed by the supplier for nutritional components and environmental contaminants. The lot number and expiration date of each batch of feed used during the study were recorded. The tap water was purified by reverse osmosis and ultraviolet light irradiation and supplied to the animals by an automatic watering

system or water bottles (nesting boxes). Water supplying the facility is analyzed for contaminants according to SLI Standard Operating Procedures. Within generally accepted limits, there were no contaminants in the diet or drinking water which would interfere with the conduct of the study.

#### 9.3.4. Environmental Conditions

The environmental controls in the animal room were set to maintain room temperature and relative humidity ranges of 65 to 79°F (18 to 26°C) and 30 to 70%, respectively. Environmental control equipment was monitored and adjusted as necessary to minimize fluctuations in the animal room environment. Light timers were set to maintain a 12-hour light/12-hour dark cycle and the room ventilation was set to produce 10 to 15 air changes per hour. The room temperature and relative humidity were recorded a minimum of once daily and were within range on each day of the study.

#### 9.4. Experimental Procedures for the Toxicity Study

#### 9.4.1. Study Group Design

The following table presents the study group design and dosage levels tested:

	No. o	f Adult		Dosage	Dosage	Dosage
	Animals		Dosage.	Level	Conc.	Volume
Group	Male	Female	Material	(mg/kg/day)	(mg/mL)	(mL/kg)
1	10	5	Corn Oil	0	0	2
2	10	5	Acetophenone	75	37.5	2
_ 3	10	5	Acetophenone	225	112.5	2
4	10	5	Acetophenone	750	375	2
Note: After a minimum of 14 days of treatment, males were cohabited with						

females in the reproduction/developmental screening study.

# 9.4.2. Rationale for Dosage Level Selection

Dosage levels were selected by the Sponsor and Study Director in an attempt to produce graded responses to the test article. The high-dose level was expected to produce some toxic effects, but not excessive lethality. The mid-dose level was expected to produce no to minimal observable effects and the low-dose level was expected to produce no observable effects.

# 9.4.3. Randomization and Group Assignment

On day -1, the animals were weighed and examined in detail for signs of physical disorder (detailed clinical observations). Animals determined to be suitable test subjects were randomly assigned to groups using a computer randomization program. The program ranked the animals according to day -1 body weights and randomly assigned the animals to study groups in a stratified block design. Disposition of animals not assigned to the study was documented in the study records. On the day following receipt, animals were approximately eight weeks of age with body weights ranging from 246 to 285 grams for males and 164 to 191 grams for females.

#### 9.4.4. Treatment

Dosing preparations were administered orally, by gavage, as a single dose daily. The animals were dosed for a minimum of four weeks, up to and including the day prior to scheduled euthanasia. All doses were administered using a plastic syringe with an attached gavage needle. Individual doses were based on the most recent body weight data.

### 9.5. Parameters Evaluated for the Toxicity Study

#### 9.5.1. Clinical Observations

Mortality/general health checks were performed twice daily, in the morning and afternoon. Cage-side observations for overt signs of toxicity were performed once daily within approximately one-half hour to two hours following dosing. Detailed clinical observations were performed a minimum of weekly and on the day of scheduled euthanasia.

#### 9.5.2. Functional Observation Battery

An abbreviated functional observation battery (FOB), including home cage, removal from home cage and open field evaluation, was performed for all animals beginning on day -1 and weekly thereafter (days 7, 14 and 21). A full FOB assessment, including home cage, removal from home cage, open field evaluation, manipulative tests and motor activity measurements, was performed following 28 days of treatment. On day 28, observations for home cage, removal from home cage and open field evaluation were recorded for all animals. On day 29, manipulative tests were performed for ten males each in groups 1 and 2, five males each in groups 3 and 4 and all females. On day 30, motor activity measurements were performed for five males and five females from each group. Definitions of the grades assigned to each FOB observation are presented in Appendix D. The following parameters were evaluated:

#### 9.5.2.1. Home Cage Observations

Body posture Clonic involuntary motor movements Tonic involuntary motor movements Vocalization

#### 9.5.2.2. Removal from Home Cage Observations

Ease of removal
Reactivity to being handled
Ocular discharge
Eyelid closure
Salivation
Piloerection

#### 9.5.2.3. Open Field Observations

Clonic involuntary motor movements Tonic involuntary motor movements Gait score Gait abnormalities -Mobility score
Arousal
Stereotypy
Bizarre behavior
Urination
Defecation
Rearing

#### 9.5.2.4. Manipulative Tests

Approach response
Touch response
Startle response
Tail pinch
Pupil response
Righting ability
Forelimb grip strength
Hindlimb grip strength
Landing foot splay
Body temperature

#### 9.5.2.5. Motor Activity

Each animal was evaluated for behavioral changes in an open field chamber (San Diego Instruments, San Diego, California). For each animal, the test consisted of an one-hour observation period in the open field chamber. Data included the total number of squares entered during the testing interval. A white noise generator (SPER Scientific Sound Meter Model 840029) was activated prior to open field testing to reduce auditory disturbance to the animals. The white noise sound level in each enclosure ranged from 74 to 75 dB prior to motor activity measurements. In addition, the motor activity test was performed under red lighting to reduce observational disturbances to the animals. Light readings for each enclosure prior to testing ranged from 0.30 to 0.33 foot candles.

#### 9.5.3. Body Weights

Individual body weights were recorded on days 0, 3, 7, 12, 16, 20, 23, 27 and 30. A final body weight was recorded prior to scheduled euthanasia (day 33/34).

#### 9.5.4. Food Consumption

Individual food consumption was recorded on the same days as body weights (except for males during cohabitation with females in the reproduction/developmental screening study).

#### 9.5.5. Clinical Pathology

Blood was collected from five males and five females from each group on the day of scheduled euthanasia (day 33 for males and day 34 for females) for evaluation of selected hematology, coagulation and clinical chemistry parameters. Blood samples for hematology and clinical chemistry parameters were obtained via the orbital plexus while the animals were under light isoflurane anesthesia and blood samples for coagulation parameters were obtained from the vena cava following carbon dioxide inhalation at scheduled euthanasia. Feed was withheld overnight prior to blood collection; however, water was available.

Clinical pathology methods are presented in Appendix E. The following parameters were evaluated:

### 9.5.5.1. Hematology

Erythrocyte count (RBC)

Hematocrit (Hct)

Hemoglobin concentration (Hgb)

Mean corpuscular hemoglobin (MCH)

Mean corpuscular hemoglobin concentration (MCHC)

Mean corpuscular volume (MCV)

Platelet count

Total and differential leukocyte counts

#### 9.5.5.2. Coagulation

Prothrombin time (PT)

Activated partial thromboplastin time (APTT)

#### 9.5.5.3. Clinical Chemistry

Alanine aminotransferase (ALT)

Albumin

Albumin/globulin ratio (calculated)

Alkaline phosphatase

Aspartate aminotransferase (AST)

Calcium

Cholesterol

Creatinine

Globulin (calculated)

Glucose

Electrolytes (sodium, potassium and chloride)

Phosphorus

Total bilirubin

Total serum protein

Triglycerides

Urea nitrogen

#### 9.5.6. Scheduled Euthanasia and Necropsy

All animals were subjected to a complete gross necropsy at scheduled euthanasia. The necropsy examination included examination of the external surfaces of the body, all orifices, and the cranial, thoracic, abdominal and pelvic cavities and their contents. The males were euthanized on day 33 and the females were euthanized on day 34 by carbon dioxide inhalation followed by exsanguination. All abnormalities were recorded. The animals were fasted overnight prior to euthanasia.

Fresh organ weights were obtained at scheduled euthanasia for the adrenals, brain, epididymides, heart, kidneys, liver, spleen, testes and thymus. Paired organs were weighed together. The following organs and tissues were preserved in 10% neutral buffered formalin for possible future histopathological examination:

Accessory genital organs (epididymides, seminal vesicles and prostate or uterus and vagina)

Adrenals

All gross lesions

Aorta

Brain (including sections of medulla/pons, cerebellar cortex and cerebral cortex)

Cecum

Colon

Duodenum

Esophagus

Exorbital lachrymal glands

Eyes with optic nerve

Femur (including articular surface) and bone marrow

Heart

lleum

Jejunum

Kidneys

Liver (three sections collected)

Lungs (infused with formalin) with bronchi

Mammary gland

Mandibular lymph node

Mediastinal lymph node

Mesenteric lymph node

Ovaries

Pancreas

Peripheral nerve (sciatic)

Pituitary

Rectum

Skeletal muscle (thigh)

Skin

Spinal cord (cervical, midthoracic and lumbar)

Spleen

Sternum with bone marrow

Stomach (glandular/nonglandular)

Submaxillary salivary gland

Testes (preserved in Bouin's fixative for 48 to 96 hours, rinsed and then retained in 70% ethanol)

**Thymus** 

Thyroid/parathyroid

Tongue

Trachea

Urinary bladder

# 9.5.7. Histopathology

All tissues and organs collected at necropsy from five males and five females from each group and gross lesions from all animals were processed for histopathological examination. The tissues were trimmed, embedded in paraffin, sectioned and stained with hematoxylin

and eosin. Histology was performed by HistoTechniques, Powell, Ohio, and the tissues were examined microscopically by Dr. William H. Baker, a board certified veterinary pathologist.

# 9.6. Experimental Procedures for the Reproduction/Developmental Screening Study

#### 9.6.1. Study Group Design

The following table presents the study group design and dosage levels tested:

Group	No. of Females	Dosage Material	Dosage Level (mg/kg/day)	Dosage Conc. (mg/mL)	Dosage Volume (mL/kg)
1	10	Corn Oil	0	0	2
2	10	Acetophenone	75	37.5	2
3	10	Acetophenone	225	112.5	2
4	10	Acetophenone	750	375	2

#### 9.6.2. Rationale for Dosage Level Selection

Dosage levels were selected by the Sponsor and Study Director in an attempt to produce graded responses to the test article. The high-dose level was expected to produce some toxic effects, but not excessive lethality. The mid-dose level was expected to produce no to minimal observable effects and the low-dose level was expected to produce no observable effects.

### 9.6.3. Randomization and Group Assignment

On day -1, the animals were weighed and examined in detail for signs of physical disorder (detailed clinical observations). Animals determined to be suitable test subjects were randomly assigned to groups using a computer randomization program. The program ranked the animals according to day -1 body weights and randomly assigned the animals to study groups. Disposition of animals not assigned to the study was documented in the study records. On the day following receipt, the animals were approximately eight weeks of age with body weights ranging from 162 to 201 grams.

#### 9.6.4. Treatment

Dosing preparations were administered orally, by gavage, as a single dose daily. The animals were dosed for a minimum of 14 days prior to mating and continuing through lactation day 3. All doses were administered using a plastic syringe with an attached gavage needle. Individual doses were based on the most recent body weight data.

# 9.7. F0 Parameters Evaluated for the Reproduction/Developmental Screening Study

# 9.7.1. F0 Clinical Observations

Mortality/general health checks were performed twice daily, in the morning and afternoon. Cage-side observations for overt signs of toxicity were performed once daily within approximately one-half hour to two hours following dosing. Detailed clinical observations were performed a minimum of weekly until evidence of mating was detected, daily during gestation and lactation and on the day of scheduled euthanasia

#### 9.7.2. F0 Body Weights

Individual body weights were recorded on days 0, 3, 7 and 12 prior to mating. When positive evidence of mating was detected, the females were weighed on gestation days 0, 7, 14 and 20. Following parturition, the females were weighed on lactation days 1 and 4. Females without evidence of mating were weighed twice weekly until euthanasia.

#### 9.7.3. F0 Food Consumption

Individual food consumption was recorded on the same days as body weights (except during cohabitation).

#### 9.7.4. F0 Breeding

Following a minimum of 14 days of treatment for the F0 females, each female designated for the mating phase was cohabited with a single male from the same treatment group (1:1 pairing) in the toxicity study. Each mating pair was observed daily for evidence of copulation. Evidence of mating was determined by the presence of a copulatory plug in the vagina or a sperm positive vaginal smear. The day evidence of copulation was confirmed was designated as day 0 of gestation and the female was returned to her cage. If no evidence of copulation was observed after 14 days of mating, the female was separated from the male and the mating phase was concluded.

#### 9.7.5. F0 Parturition and Lactation

On approximately gestation day 18, females with confirmed copulation were transferred to individual plastic boxes containing nesting material. Each F0 female was observed for signs of parturition a minimum of twice daily. The time parturition was first detected and the time parturition was completed were recorded, when possible. Signs of difficult or prolonged delivery were recorded, if observed. The day on which parturition was complete was designated as lactation day 0. The females and their offspring remained together until in-life conclusion on lactation day 4. Abnormal nursing and nesting behaviors were recorded, if observed. The offspring were designated as the F1 generation. F0 females with no evidence of mating were examined for signs of parturition beginning 19 days following initiation of the mating phase.

# 9.7.6. F0 Euthanasia and Necropsy

F0 females were euthanized by carbon dioxide inhalation followed by exsanguination and subjected to a complete gross necropsy examination. F0 females that delivered were euthanized on lactation day 4. Females that failed to deliver were euthanized 25 days after

evidence of mating was first detected (post breeding day 25). F0 females with no evidence of mating were euthanized 25 days after completion of the mating period (post breeding period day 25). Females with total litter loss were euthanized and necropsied on the day that no surviving pups remained. The necropsy examination included examination of the external surfaces of the body, all orifices, and the cranial, thoracic, abdominal and pelvic cavities and their contents. Uterine contents were examined and the number of implantation sites and number of corpora lutea (per ovary) were recorded. Uteri with no macroscopic evidence of implantation were opened and placed in 10% aqueous ammonium sulfide solution for detection of implantation sites as described by Salewski [1]. All abnormalities were recorded. The following organs and tissues were preserved in 10% neutral buffered formalin for possible future histopathological examination:

Accessory genital organs (uterus and vagina)

Adrenals

All gross lesions

Aorta

Brain (including sections of medulla/pons, cerebellar cortex and cerebral cortex)

Cecum

Colon

Duodenum

Esophagus

Exorbital lachrymal glands

Eyes with optic nerve

Femur (including articular surface) and bone marrow

Heart

lleum

Jejunum

Kidneys

Liver (three sections collected)

Lungs (infused with formalin) with bronchi

Mammary gland

Mandibular lymph node

Mediastinal lymph node

Mesenteric lymph node

**Ovaries** 

Pancreas

Peripheral nerve (sciatic)

Pituitary

Rectum

Skeletal muscle (thigh)

Skin

Spinal cord (cervical, midthoracic and lumbar)

Spleen

Sternum with bone marrow

Stomach (glandular/nonglandular)

Submaxillary salivary gland

Thymus

Thyroid/parathyroid Tongue Trachea Urinary bladder

# 9.8. F1 Parameters Evaluated for the Reproduction/Developmental Screening Study

# 9.8.1. F1 Pup Identification, Lactation Observations and Offspring Parameters

On lactation day 0, pups in each litter were individually identified using a toe marking (tattooing) system. The following parameters were recorded for each pup during lactation:

Viability: daily from days 0 to 4

External Examinations: days 0 and 4

Sex Determinations: days 0 and 4

Body Weights: days 1 and 4

# 9.8.2. F1 Unscheduled Deaths

F1 pups that were stillborn or died were subjected to a gross necropsy examination, with emphasis on developmental morphology. All internal gross lesions (except atelectasis; and scabbing, subcutaneous hemorrhage or other lesions resulting from apparent cannibalism) were preserved in 10% neutral buffered formalin for possible future histopathological examination.

#### 9.8.3. F1 Scheduled Euthanasia

All surviving pups were euthanized on lactation day 4 by an intraperitoneal injection of sodium pentobarbital and examined macroscopically for structural abnormalities or other pathological changes. During the examination, special attention was paid to the organs of the reproductive system. All gross lesions were preserved in 10% neutral buffered formalin for possible future histopathological examination.

#### 10. STATISTICAL ANALYSES

Statistical analyses for the toxicity study were performed using the SLI Alpha GenTox computer system. Data, including body weights, body weight changes and food consumption were analyzed by One-Way Analysis of Variance (ANOVA) [2]. If significance was detected with ANOVA (p<0.05), pair wise group comparisons proceeded using the Tukey-Kramer test [3]. Descriptive (categorical) and quanta data were analyzed by Fisher's Exact Test [4]. When significance was observed, group by group comparisons were performed using Fisher's Exact Test. Absolute and relative organ weights, and clinical pathology data were analyzed for homogeneity of variance using Levene's test [5]. If significance was detected with Levene's test (p<0.01), multiple group comparisons proceeded using the Kruskal-Wallis non-parametric ANOVA, followed by Dunn's test [6],

when p<0.05. If significance was not detected with Levene's test, parametric procedures were used to analyze the data, i.e., ANOVA followed by Tukey-Kramer test when p<0.05. All analyses were two-tailed with a minimum significance level of 5% (p<0.05).

Statistical analysis for the reproduction/developmental screening study were performed using the SLI Alpha ReproTox computer system. Data, including body weights, body weight changes, food consumption and mean live litter size were analyzed by ANOVA [7]. If significance was observed with ANOVA, control to treatment group comparisons were performed using Dunnett's test [8,9]. Count data were analyzed using R x C Chi-Square test [10] followed by Fisher's Exact Test [11] for copulation and fertility indices, pup sex ratios, the number of live and dead pups per group (on lactation day 0) and pup survival (after lactation day 0). All analyses were two-tailed with a minimum significance level of 5% (p<0.05).

# 11. MAINTENANCE OF RAW DATA, RECORDS AND SPECIMENS

The remaining test article will be returned to the Sponsor following completion of all scheduled studies with this material. All original paper data, magnetically encoded records, wet tissues, tissue blocks, slides and the final report will be transferred to the SLI archives and stored for a minimum of ten years. The Sponsor will be consulted prior to final disposition of these materials.

#### 12. RESULTS

# 12.1. Analytical Chemistry

Appendix C (SLI Analytical Chemistry Report)

Results of the homogeneity analyses revealed that the average recoveries of the 37.5 and 375 mg/mL dosing formulations were within 4.9% of the nominal concentration, indicating that the formulations were homogeneous. Results of the stability analyses revealed that the 37.5 and 375 mg/mL dosing formulations were stable for 10 days following room temperature storage. The average results of the concentration verification analyses were within 5.3% of the nominal concentrations. No Acetophenone was detected in the vehicle control samples analyzed during the course of the study.

### 12.2. Toxicity Study

# 12.2.1. Survival and Clinical Observations

Table 1 (Summary Data)
Appendix F (Individual Data)

All males and females survived to scheduled euthanasia at the study termination.

Clinical signs were observed for males and females in the 225 and 750 mg/kg/day groups. In males, the clinical signs included a low incidence of predose salivation and postdose

salivation in the 225 mg/kg/day group, and predose and postdose salivation, postdose wobbly gait and a low incidence of postdose urine stain in the 750 mg/kg/day group. No remarkable clinical signs were observed for males in the 75 mg/kg/day group.

In females, clinical signs included a low incidence of urine stain and postdose salivation in the 225 mg/kg/day group, and hairloss, urine stain, predose and postdose salivation and postdose wobbly gait in the 750 mg/kg/day group. Additional clinical signs for one female (#1827) in the 750 mg/kg/day group included a low incidence of dark material around the nose, urine stain, ocular discharge, impaired mobility, cool to the touch, increased activity and nasal discharge following dosing. Remarkable clinical signs for females in the 75 mg/kg/day group were limited to a single incidence of postdose salivation in one female.

#### 12.2.2. Functional Observation Battery

Table 2 (Summary Data)
Appendix G (Individual Data)

Mean forelimb grip strength and mean motor activity of males in the 750 mg/kg/day group were statistically lower than controls on day 29. No other toxicologically meaningful differences were noted in the functional observation battery. Body posture of males in the 75 mg/kg/day group and females in the 750 mg/kg/day group was statistically different than controls on day 21. These latter differences were not considered toxicologically meaningful since they did not follow a consistent pattern or dose response.

# 12.2.3. Body Weights and Body Weight Changes

Tables 3 and 4 (Summary Data)
Appendices H and I (Individual Data)

Statistically significant body weight loss occurred for males in the 750 mg/kg/day group during days 0-3. No other statistically significant differences in mean body weight change were noted for males or females in the 75, 225 or 750 mg/kg/day groups during the study. However, there was an apparent mean body weight loss noted in all dose groups, including the control from days 12-16. The mean body weight loss in males ranged from 19-26 grams and in the females it ranged from 5-17 grams. A compensatory rebound weight gain was noted during the following body weight change interval (days 16-20), where animals in all dose groups gained a relatively large amount of body weight, which ranged in the males from 22-36 grams and in the females from 10-25 grams.

There were no statistically significant differences in mean body weights between the control, 75, 225 and 750 mg/kg/day groups. However, mean body weights of males in the 750 mg/kg/day group were approximately 5-10% lower than controls beginning on day 3 and continuing through day 30. Mean body weights of males in the 75 and 225 mg/kg/day groups and females in the 75, 225 and 750 mg/kg/day groups were comparable to controls (within 3% of controls) throughout the study.

#### 12.2.4. Food Consumption

Table 5 (Summary Data)
Appendix J (Individual Data)

Mean food consumption (grams/animal/day) of males and females in the 750 mg/kg/day group was statistically lower than controls during study days 0-3. In addition, mean food consumption appeared to be slightly, but not statistically lower than controls for females in the 225 mg/kg/day group during days 0-3 and males in the 750 mg/kg/day group during days 16-30. No other toxicologically meaningful differences in mean food consumption were noted between the control, 75, 225 and 750 mg/kg/day groups during the study.

#### 12.2.5. Clinical Pathology

Tables 6 and 7 (Summary Data)
Appendices K and L (Individual Data)
Appendix FF (SLI Historical Control Data)

#### 12.2.5.1. Hematology and Coagulation

There were no toxicologically meaningful differences between the control, 75, 225 and 750 mg/kg/day groups in the hematology and coagulation parameters evaluated at study termination (days 33/34). A few statistically significant differences were noted; however, none of the differences were considered toxicologically meaningful since they did not follow a consistent pattern and the mean values remained well within the range of SLI historical control data.

In males, statistically significant differences in hematology and coagulation data included a lower mean prothrombin time in the 75 mg/kg/day group and a higher mean MCV value in the 750 mg/kg/day group compared to controls. In females, statistically significant differences in hematology and coagulation data were limited to lower mean erythrocyte and hematocrit values in the 750 mg/kg/day group compared to controls.

#### 12.2.5.2. Clinical Chemistry

Several statistically significant differences in biochemistry data were noted between the control, 75, 225 and 750 mg/kg/day groups at study termination (days 33/34). However, with the exception of higher mean total protein, calcium and cholesterol for males in the 750 mg/kg/day group and higher mean cholesterol for females in the 750 mg/kg/day group, the mean values remained within the range of SLI historical control data. The toxicological significance of the higher total protein and calcium in the 750 mg/kg/day males and higher cholesterol in 750 mg/kg/day males and females is not clear; however, it should be noted that the mean values for these parameters were outside the range of SLI historical control data.

Additional statistically significant differences in male biochemistry data included higher mean total protein, albumin, calcium and phosphorus in the 225 mg/kg/day group and higher albumin, globulin, glucose, sodium, potassium and phosphorus in the 750 mg/kg/day group compared to controls. In females, additional statistically significant differences in biochemistry data included higher mean total bilirubin the 75 mg/kg/day group, higher mean

total protein, albumin and globulin in the 225 mg/kg/day group, and higher mean ALT, total protein, albumin and potassium in the 750 mg/kg/day group compared to controls. The mean values of these additional differences were within the range of SLI historical control data. Even though these values were within the historical control range, a common finding to both male and female animals included a dose-related increase in total protein, albumin and globulin that appeared to correlate with a dose-related increase in liver weight. These changes may be related to a compensatory reaction to the test article, possibly due to its metabolism.

### 12.2.6. Gross Necropsy Observations

Table 8 (Summary Data) Appendix M (Individual Data)

No remarkable findings were noted for males or females in the control, 75, 225 or 750 mg/kg/day groups at scheduled necropsy.

# 12.2.7. Organ Weights

Tables 9 and 10 (Summary Data) Appendix N (Individual Data)

There were no toxicologically meaningful differences in absolute or relative organ weights between the control, 75, 225 and 750 mg/kg/day groups. A few statistically significant differences in organ weights were noted; however, the findings did not correlate with any abnormal biochemistry or histopathology.

Statistically significant differences in organ weight data included lower absolute heart and epididymides weight and higher liver weight relative to final body weight for males in the 750 kg/kg/day group, and higher absolute liver weight and liver weight relative to final body weight and higher kidney weight relative to final body weight for females in the 225 and 750 mg/kg/day groups compared to controls.

#### 12.2.8. Histopathology

Appendix O (Histopathology Report)

No toxicologically significant test article-related lesions were noted in any of the tissues examined. A minimal to mild hyaline droplet nephropathy was observed for males in the 75, 225 and 750 mg/kg/day groups. The hyaline droplets were sometimes accompanied by minimal tubular epithelial degeneration and regeneration. Hyaline droplets can be induced by a variety of chemical compounds in this strain of male rats and is frequently associated with the accumulation of  $\alpha_{2\nu}$ -globulin within the hyaline droplets [12]. However, this finding is not considered toxicologically significant for humans. Minimal to mild vacuolar change was observed in individual hepatocytes in males from the 750 mg/kg/day group. This change was interpreted as a background lesion based on the nature and distribution of changes. In addition, similar changes have been seen in control animals in other toxicity studies. Other microscopic changes were considered to be normal findings of rats of this age and strain.

# 12.3. Reproduction/Developmental Screening Study

# 12.3.1. F0 Survival and Clinical Observations

Table 11 (Summary Data)
Appendix P (Individual Data)

No mortality occurred in the F0 females. One F0 female in the control group (#1855) with no evidence of mating was euthanized on postbreeding period day 25. Two F0 females in the control group (#1867 and #1870), one F0 female in the 75 mg/kg/day group (#1869) and one F0 female in the 750 mg/kg/day (#1842) with evidence of mating failed to deliver and were euthanized on postbreeding day 25. Six F0 females in the 750 mg/kg/day group (#1841, #1847, #1848, #1857, #1861 and #1876) were euthanized due to total litter loss. These females were euthanized on lactation days 1-4, the day all pups in the litter were found dead. All other F0 females in the control, 75, 225 and 750 mg/kg/day groups survived to scheduled euthanasia on lactation day 4.

Clinical signs were observed primarily for females in the 225 and 750 mg/kg/day groups. The clinical signs included a low incidence of urine stain and predose salivation, a low incidence of postdose feces small in size and postdose salivation in the 225 mg/kg/day group, and a low incidence of decreased activity, skin pale in color, unkempt appearance, rough coat, postdose dark material around nose, postdose few feces, and predose and postdose salivation and postdose wobbly gait in the 750 mg/kg/day group. In addition, one female in the 750 mg/kg/day group (#1851) appeared to have prolonged parturition. Remarkable clinical signs for females in the 75 mg/kg/day group were limited to a single incidence of urine stain in two females.

# 12.3.2. F0 Body Weights and Body Weight Changes

Tables 12-17 (Summary Data) Appendices Q-V (Individual Data)

Mean body weight change of F0 females in the 750 mg/kg/day group was statistically lower than controls during gestation days 0-7. No other statistically significant differences in mean body weight change were noted in the F0 females prior to mating or during gestation and lactation. However, F0 females in the 225 and 750 mg/kg/day groups lost slightly more body weight than controls during days 0-3 (prior to mating) and mean body weight change of F0 females in the 750 mg/kg/day group appeared to be slightly lower than controls during lactation days 1-4.

There were no statistically significant differences in mean body weights between F0 females in the control, 75, 225 and 750 mg/kg/day groups. However, mean body weight of F0 females in the 750 mg/kg/day group was slightly (approximately 6%) lower than controls on gestation day 7. At all other intervals, mean body weights of F0 females in the 750 mg/kg/day group were within approximately 4% of controls. Mean body weights of F0 females in the 75 and 225 mg/kg/day groups were within 4% of controls prior to mating and during gestation and lactation.

### 12.3.3. F0 Food Consumption

Tables 18-20 (Summary Data)
Appendices W-Y (Individual Data)

Mean food consumption (grams/animal/day) of F0 females in the 225 and 750 mg/kg/day groups was statistically lower than controls during days 0-3. In addition, mean food consumption appeared to be slightly, but not statistically lower than controls for F0 females in the 750 mg/kg/day group during gestation days 0-7 and lactation days 1-4. No other toxicologically meaningful differences in mean food consumption were noted between the control, 75, 225 and 750 mg/kg/day groups.

# 12.3.4. F0 Female Reproduction Indices, Gestation Lengths and F1 Pup Viability

Table 21 (Summary Data)
Appendices Z and AA (Individual Data)
Appendix FF (SLI Historical Control Data)

The F0 female mating index was 100% in the control, 75, 225 and 750 mg/kg/day groups. The F0 female fertility index was 77.8% in the control group, 90% in the 75 and 750 mg/kg/day groups and 100% in the 225 mg/kg/day group. The F0 mean gestation length was 21.9 days in the control group and 22.0 days in the 75, 225 and 750 mg/kg/day groups. A total of seven females in the control group, nine females each in the 75 and 750 mg/kg/day groups and ten females in the 225 mg/kg/day groups completed delivery. However, two females in the control group, four females in the 75 mg/kg/day group, three females in the 225 mg/kg/day group and seven females in the 750 mg/kg/day group had stillborn pups.

The mean number of F1 pups delivered was comparable in the control, 75, 225 and 750 mg/kg/day groups. However, the live birth index (number of liveborn pups/total number of pups delivered) was statistically lower in the 750 mg/kg/day group compared to controls. In addition, the number of stillborn pups in the 750 mg/kg/day group was statistically higher than controls.

A total of five F1 pups in the 750 mg/kg/day group was born alive and died later on lactation day 0. In addition, 79 pups in the 750 mg/kg/day group died or were missing and/or cannibalized during lactation days 1-4 compared to 6 pups in the control group, 5 pups in the 75 mg/kg/day group and 8 pups in the 225 mg/kg/day group. A total of six litters with live born pups had no live pups remaining on lactation day 4 (total litter loss) in the 750 mg/kg/day group. The number of pups surviving to lactation day 4 was 99 in the control group, 131 in the 75 mg/kg/day group, 137 in the 225 mg/kg/day group and 25 in the 750 mg/kg/day group. The viability index on lactation day 4 was 94.3% in the control group, 96.3% in the 75 mg/kg/day group, 94.5% in the 225 mg/kg/day group and 22.9% in the 750 mg/kg/day group. The number of F1 pups dying, missing and/or cannibalized during lactation days 1-4, the number of litters with total litter loss and the number of surviving pups on lactation day 4 in the 750 mg/kg/day group were statistically different from controls.

The mean live pups/litter in the 750 mg/kg/day group was slightly, but not statistically lower than controls on lactation day 0 and statistically lower than controls on lactation days 1-4. F1 pup viability in the 75 and 225 mg/kg/day groups was comparable to controls on lactation days 0-4. The F1 pup sex ratio was comparable between the control, 75, 225 and 750 mg/kg/day groups on lactation days 0 and 4.

### 12.3.5. F1 Pup Body Weights during Lactation

Table 21 (Summary Data)
Appendix BB (Individual Data)
Appendix FF (SLI Historical Control Data)

Mean F1 pup weights in the 750 mg/kg/day group were statistically lower than controls on lactation days 1 and 4, and outside the range of SLI historical control data. Mean F1 pup weights in the 75 and 225 mg/kg/day groups were comparable to controls on lactation days 1 and 4.

#### 12.3.6. F1 Pup Observations during Lactation

Table 22 (Summary Data) Appendix CC (Individual Data)

Remarkable F1 pup observations during lactation days 0-4 included desquamation in the 225 and 750 mg/kg/day groups and a low incidence of gasping and skin pale in color, cool to the touch, skin with shiny appearance and skin appears tight – restricts movement in the 750 mg/kg/day group. No remarkable F1 pup observations were noted in the 75 mg/kg/day group.

#### 12.3.7. F1 Pup Gross Necropsy Observations

Table 23 (Summary Data) Appendix DD (Individual Data)

Remarkable gross necropsy observations for F1 pups stillborn included a single incidence of cleft palate, a single incidence of edema, increased incidences of atelectasis, milk not present in the stomach, and dermal hypoplasia in the 750 mg/kg/day group. No remarkable gross necropsy findings were noted for F1 pups stillborn in the 75 and 225 mg/kg/day groups.

Remarkable gross necropsy findings for F1 pups born alive but found dead during lactation days 0-4 included scabbing, desquamation, dermal hypoplasia and an increased incidence of milk not present in the stomach in the 750 mg/kg/day group. In addition, autolysis was observed for 22 F1 pups found dead in the 750 mg/kg/day group. No remarkable gross necropsy findings were noted for F1 pups found dead in the 75 and 225 mg/kg/day groups.

Remarkable gross necropsy findings for F1 pups at scheduled euthanasia on lactation day 4 included scabbing and desquamation in the 225 and 750 mg/kg/day groups. No remarkable gross necropsy findings were noted for surviving F1 pups in the 75 mg/kg/day group.

# 12.3.8. F0 Maternal Necropsy Observations

Table 24 (Summary Data)
Appendix EE (Individual Data)

Two F0 females in the 750 mg/kg/day group with total litter loss (#1857 and #1861) had pup tissue mixed with ingesta in the stomach. No other remarkable findings were noted at necropsy for F0 females in the control, 75, 225 or 750 mg/kg/day groups.

#### 13. DISCUSSION

This study was conducted to: (1) provide initial information on the repeated-dose systemic toxicity of Acetophenone, with emphasis on potential neurological effects, and (2) serve as a screening study for potential reproductive and developmental effects in male and female rats.

#### 13.1. Toxicity Study

No mortality occurred in the toxicity study. Clinical signs were observed for males and females in the 225 and 750 mg/kg/day groups. The clinical signs included urine stain, predose salivation and postdose salivation in the 225 and 750 mg/kg/day groups, and postdose wobbly gait and postdose urine stain in the 750 mg/kg/day group. Remarkable clinical signs in the 75 mg/kg/day group were limited to a single incidence of postdose salivation in one female. Mean forelimb grip strength and mean motor activity of males in the 750 mg/kg/day group were statistically lower than controls on day 29. No other toxicologically meaningful differences were noted in the functional observation battery.

Statistically significant body weight loss occurred for males in the 750 mg/kg/day group during days 0-3. An apparent body weight loss was noted in all dose groups for both male and female animals during days 12-16 and this was followed by a compensatory rebound in weight gain the following body weight change interval (days 16-20). The definitive reason for this occurrence could not be determined, but it may be related to the initiation of mating. There were no statistically significant differences in mean body weights; however, mean body weights of males in the 750 mg/kg/day group were approximately 5-10% lower than controls on days 3-30. No other toxicologically meaningful differences in mean body weights or body weight change were noted during the study.

Mean food consumption of males and females in the 750 mg/kg/day group was statistically lower than controls during study days 0-3. In addition, mean food consumption appeared to be slightly, but not statistically lower than controls for females in the 225 mg/kg/day group during days 0-3 and males in the 750 mg/kg/day group during days 16-30. No other toxicologically meaningful differences in mean food consumption were noted during the study.

There were no toxicologically meaningful differences between the control, 75, 225 and 750 mg/kg/day groups in the hematology and coagulation parameters evaluated at study termination. With the possible exception of higher mean total protein, calcium and cholesterol for males in the 750 mg/kg/day group and higher mean cholesterol for females in

the 750 mg/kg/day group, there were no toxicologically meaningful differences in the biochemistry parameters evaluated. The toxicological significance of the higher total protein, calcium and cholesterol values is not clear; however, the mean values for these parameters were outside the range of SLI historical control data. There was a common finding to both male and female animals that included a dose-related increase in total protein, albumin and globulin that appeared to correlate with a dose-related increase in liver weight. These changes may be related to a compensatory reaction to the test article, possibly due to its metabolism.

No remarkable gross necropsy findings were noted in the control, 75, 225 or 750 mg/kg/day groups and there were no toxicologically meaningful differences in absolute or relative organ weights between the groups. No toxicologically significant test article-related microscopic lesions were noted in any of the tissues examined. A minimal to mild hyaline droplet nephropathy was observed for males in the 75, 225 and 750 mg/kg/day groups; however, this finding is not considered toxicologically significant for humans [12].

#### 13.2. Reproductive/Developmental Study

No mortality occurred for the F0 females in the reproduction/developmental screening study. Clinical signs were observed for F0 females in the 225 and 750 mg/kg/day groups. The clinical signs included urine stain, predose salivation, postdose feces small in size and postdose salivation in the 225 mg/kg/day group, and decreased activity, skin pale in color, unkempt appearance, rough coat, postdose dark material around nose, postdose few feces, predose and postdose salivation and postdose wobbly gait in the 750 mg/kg/day group. In addition, one female in the 750 mg/kg/day group appeared to have prolonged parturition. Remarkable clinical signs for females in the 75 mg/kg/day group were limited to a single incidence of urine stain in two females.

Mean body weight change of F0 females in the 750 mg/kg/day group was statistically lower than controls during gestation days 0-7. There were no statistically significant differences in mean body weights; however, mean body weight of F0 females in the 750 mg/kg/day group was slightly (approximately 6%) lower than controls on gestation day 7. No other toxicologically meaningful differences in mean body weights or body weight change were noted during the study.

Mean food consumption of F0 females in the 225 and 750 mg/kg/day groups was statistically lower than controls during days 0-3. In addition, mean food consumption appeared to be slightly, but not statistically lower than controls for F0 females in the 750 mg/kg/day group during gestation days 0-7 and lactation days 1-4. No other toxicologically meaningful differences in mean food consumption were noted between the control, 75, 225 and 750 mg/kg/day groups.

The F0 female mating and fertility indices and mean gestation lengths were comparable between the control group, 75, 225 and 750 mg/kg/day groups. The mean number of F1 pups delivered was comparable in the control, 75, 225 and 750 mg/kg/day groups. However, the live birth index (number of liveborn pups/total number of pups delivered) was

statistically lower in the 750 mg/kg/day group compared to controls, and the number of stillborn pups in the 750 mg/kg/day group was statistically higher than controls.

The number of F1 pups dying, missing and/or cannibalized during lactation days 1-4 and the number of litters with total litter loss in the 750 mg/kg/day group were statistically higher than controls and the number of surviving pups on lactation day 4 in the 750 mg/kg/day group was statistically lower than controls. The mean live pups/litter in the 750 mg/kg/day group was slightly, but not statistically lower than controls on lactation day 0 and statistically lower than controls on lactation days 1-4. Mean F1 pup weights in the 750 mg/kg/day group were statistically lower than controls on lactation days 1 and 4. F1 pup viability and mean F1 pup weights in the 75 and 225 mg/kg/day groups was comparable to controls during lactation days 0-4. The F1 pup sex ratio was comparable between the control, 75, 225 and 750 mg/kg/day groups on lactation days 0 and 4.

Remarkable F1 pup observations included desquamation in the 225 and 750 mg/kg/day groups and a low incidence of gasping and skin pale in color, cool to the touch, skin with shiny appearance and skin appears tight – restricts movement in the 750 mg/kg/day group. No remarkable F1 pup observations were noted in the 75 mg/kg/day group.

Remarkable gross necropsy observations for F1 pups stillborn included a single incidence each of cleft palate and edema, increased incidences of atelectasis, milk not present in the stomach, and dermal hypoplasia in the 750 mg/kg/day group. Remarkable gross necropsy findings for F1 pups born alive but found dead during lactation days 0-4 included scabbing, desquamation, dermal hypoplasia and an increased incidence of milk not present in the stomach in the 750 mg/kg/day group. In addition, autolysis was observed for 22 F1 pups found dead in the 750 mg/kg/day. No remarkable gross necropsy findings were noted for F1 pups stillborn or found dead in the 75 and 225 mg/kg/day groups. Remarkable gross necropsy findings for F1 pups at scheduled euthanasia (lactation day 4) included scabbing and desquamation in the 225 and 750 mg/kg/day groups. No remarkable gross necropsy findings were noted for surviving F1 pups in the 75 mg/kg/day group.

Remarkable gross necropsy findings in F0 females were limited to two females in the 750 mg/kg/day group with total litter loss that had pup tissue mixed with ingesta in the stomach. No other remarkable findings were noted at necropsy for F0 females in the control, 75, 225 or 750 mg/kg/day groups.

#### . 14. CONCLUSION

Based on the results of this study, a dosage level of 75 mg/kg/day was considered a no-observed-adverse-effect level (NOAEL) for systemic toxicity. Other than possible indications of central nervous system (CNS) depression, such as wobbly gait, reduced forelimb strength and decreased motor activity in the high-dose group, there was no evidence of more severe CNS effects in this study. Thus, a dosage level of 225 mg/kg/day was considered the NOAEL for neurological effects. As a result of a significantly decreased live birth index (increased rate of stillbirths), as well as decreased pup survival during the lactation phase and a decreased mean pup body weight in the high-dose group, a dosage level of 225 mg/kg/day was considered a NOAEL for reproductive effects. However, the mating and fertility indices and mean gestation length for the test article-treated females appeared unaffected at dosage levels up to 750 mg/kg/day.

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15. REPORT REVIEW

Joseph C. Siglin, Ph.D //DABT Vice President, Managing Director Date 1.13.03

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